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ARTICLE
Demonstrating Connections Between Neuron Signaling and Behavior using *C. elegans* Learning Assays and Optogenetics in a Laboratory Class

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Due to its well-described neural circuitry and identified connectome, the *Caenorhabditis elegans* model is well-suited for demonstrating connections between neuron signaling and behavioral outcome. In the 2017 FUN workshop at Dominican University, three behavior-based techniques were introduced for their ease of introduction to students, the flexible data collection options they offer and the inexpensive cost to implement in an education setting. These behavioral assays were adapted to address some of the challenges of performing *C. elegans* behavior experiments in lab classes and included: an associative chemosensory avoidance task to examine behavior of groups of worms, a mechanosensory task to observe individual worm behavior and an optogenetics assay to directly manipulate neuron signaling and simultaneously observe resultant behavior. Methods for these assays as well as example data collected by undergraduate students in a lab class are provided. FUN Workshop feedback and assessment indicate these assays were well-received and overall seen as valuable for introducing neuroscience and behavior to undergraduates in a lab class.

Key words: associative chemotaxis; learning; biogenic amines; gustatory signaling, channelrhodopsin (ChR2); head touch; mechanosensory; habituation.

It can be challenging to find reliable protocols to introduce undergraduates to the premise that alterations in neural signaling can be measured by changes in behavior. With restrictions of laboratory class scheduling, time and facilities, as well as limited student technical expertise, a degree of protocol flexibility is needed. Thus, simplifying experiment procedures and reducing the number of extraneous variables in a behavioral task greatly facilitate lab class instruction. With these considerations in mind, for undergraduate lab classes I employ the well-understood and tractable model system *Caenorhabditis elegans*. This microscopic nematode (see Figure 1) has been well-studied, and because of its somewhat simple biology, its identified and described neural circuitry (see WormAtlas) as well as the wealth of biology tools available with this model, it is possible to easily manipulate neural signaling and monitor for resultant behavioral changes. Further, the accessibility of this model lends itself to introducing students to very sophisticated Neuroscience techniques (i.e., optogenetics) without requiring a research background.

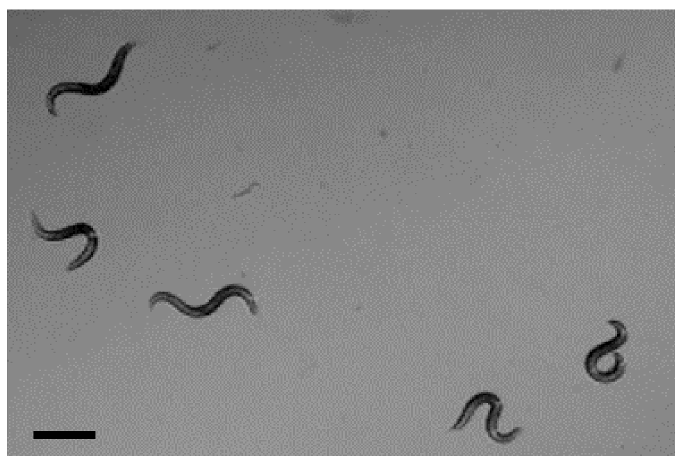

Figure 1. C. elegans viewed under EZ4 Leica dissecting microscope captured at ~100X magnification (total). The head of the worm appears lighter in color than the narrower tail. Scale bar = 0.5 mm.

C. elegans offers several strengths for use in a laboratory class (see Lemons [2016] for more details regarding advantages of using *C. elegans* in education settings). Some of the more well-known advantages of this model system include a fully mapped and sequenced genome (*C. elegans Sequencing Consortium,* 1998) as well as a community of researchers who make mutant and transgenic strains available via strain libraries (see Materials and Methods). *C. elegans* is also amenable to gene expression manipulation through RNAi knockdown (originally discovered in *C. elegans*; Fire et al., 1998), a procedure that can be easily included in a lab class as RNAi knockdown can be induced by soaking worms in or cultivating worms on food containing double-stranded RNA (Ahringer, 2006). As well, *C. elegans* are transparent allowing students to visualize fluorescent reporter constructs (e.g., green fluorescent protein; GFP; Chalfie et al., 1994) within the intact animal and to observe the effects of light-sensitive channel activation (i.e., channelrhodopsin) in the behaving animal (see Husson et al., 2013; Fang-Yen et al., 2015) similar to other organisms (e.g., Titlow et al., 2015). Finally, *C. elegans* are also affected by exposure to pharmacological agents; thus, taken together there are many accessible methods by which neuron function and signaling can be manipulated with *C. elegans.*

Despite the seemingly mundane tube-like anatomy, *C. elegans* perform several well-characterized behaviors including: moving towards a chemical attractant (chemotaxis), locomotor avoidance response to touch (mechanoavoidance), and moving toward an amenable ambient temperature (thermotaxis). The neural circuits for

these behaviors have been identified down to the individual neurons involved and the connections that they make (Figure 2) allowing researchers and students to predict how changes in signaling of particular neurons affects a specific behavior. As well, the worm is capable of learning about new stimuli (e.g., habituation) and forming associations between stimuli; for instance, when a chemical is introduced in the absence of food, worms will subsequently avoid that chemical even if it previously served as an attractant (Saeki et al., 2001). Several researchers have uncovered mechanisms of learning using this model system (see Giles et al., 2006). These advantages facilitate introducing to students how synaptic signaling modulation is reflected as changes in measurable behaviors in an accessible way.

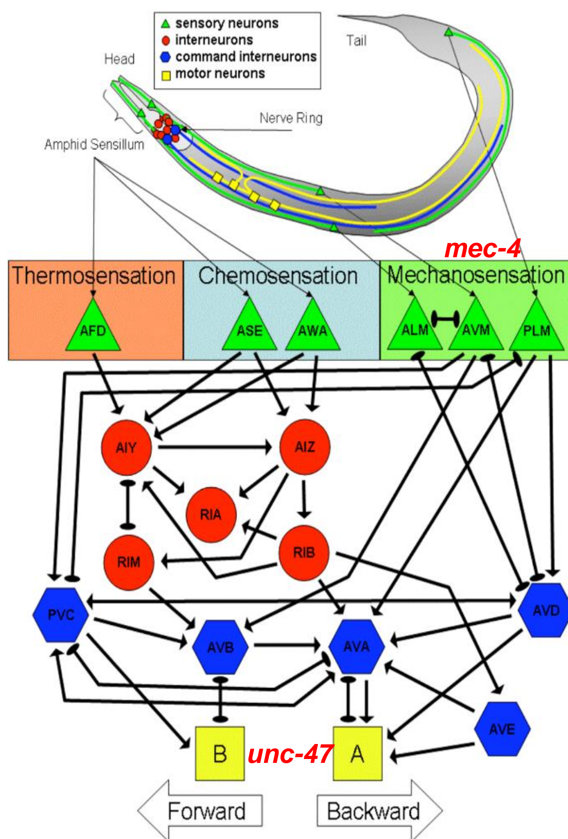

Figure 2. Identified circuits of sensory, interneuron and motor neurons involved in behavioral responses to specific types of stimuli (from Giles et al., 2006). Expression locations of genes *mec-4* and *unc-47* included for reference.

For my lab classes, students typically have one 4-hour lab session per week and will perform different behavioral assays over several lab sessions. For the 2017 FUN Workshop at Dominican University a revised version of experiments that usually take place over two lab sessions (~8 hours of instructional time) was delivered in a two-hour time period, demonstrating the time flexibility of these protocols. T he FUN workshop was divided into three parts where the first part demonstrated the use of a group behavior learning assay (associative chemoavoidance), the second part involved observing and measuring individual worm behavior with a mechanosensory assay, and the final part included manipulating neuron signaling directly with optogenetics and observing a change in response behavior. This workshop aimed to provide a design skeleton from which faculty could build larger research questions for lab classes.

MATERIALS AND METHODS

Strains

Wild-type (WT; N2 strain), *ser-3*(ad1774) deletion mutation of an octopamine receptor; ZX899 (*lite-1*(ce314); *mec-4p*::ChR2(H134R)::YFP(codon-optimized) + *unc-122p*::RFP;*glr-1p*::Mac::mCherry + *elt-2p*::GFP) provided by the *Caenorhabditis elegans* Genetics Center (CGC, University of Minnesota). Worms were maintained on OP50 (a mutated strain of *Escherichia coli* that serves as the worm's food source) on Nematode Growth Medium (NGM)-filled plates as detailed in Stiernagle (2006). All-trans retinal (ATR; R2500, Sigma) was added to OP50 (1 µL of 100mM ATR was dissolved in ethanol and added to 250 µL of OP50 suspension immediately prior to seeding) for the ChR2 strain cultivation plates. Dried plates were then wrapped in aluminum foil to prevent light exposure. All cultivation plates were maintained at room temperature.

Associative Chemotaxis

Procedures were adapted from Wormbook (Hart, 2006).

Pre-Lab preparation: Due to the 2-hour workshop time restriction, worms were pre-incubated in different experimental conditions prior to the workshop. Using low-retention pipette tips, worms were washed off cultivation plates and deposited in 1.5 mL tubes to undergo repeated washes to clean residual OP50. Once supernatant was clear, worms were resuspended and incubated for ~1 hour in one of three ¨no-food¨ conditions: wash solution (5 mM KPO4, pH 6.0, 1 mM CaCl2, 1 mM MgSO4; control), wash solution with 20 mM NaCl added or wash solution with 20 mM NaCl and 1 mM 5-HT HCl, prepared by dissolving in 0.1 M HCl for a 100 mM 5-HT HCl stock, of which 10 µL was added to 1 mL of conditioning solution.

Test Plate Preparation: 9 cm petri dishes were filled with agar in the absence of Na+ (5mM KPO4, pH 6.0, 1mM CaCl2, 1mM MgSO4, 2% agar, were mixed, autoclaved and then poured into petri dishes to dry for ~48 hours).

Procedure: First, the center 'Drop Zone' was marked on the underside of the test plate by tracing the top of a 50 mL centrifuge tube followed by adding to the center 25 µl of test solution (wash solution + 20 mM NaCl with added red food-coloring to provide an estimate of solution diffusion; see Figure 3). While the test solution was drying, incubating worm colonies were washed from tubes by brief centrifugation, afterwards aspirating the supernatant and resuspending in wash solution. Following 2 - 3 repeated washes, worms were collected by capturing ~10 - 15 µl of worm pellet from the tube bottom using low retention pipette tips cut for a larger orifice to facilitate worm collection and prevent shearing of worms. Worm pellets were then deposited onto test plates in the Drop Zone area. Participants then reported observations of worm migration (number of worms inside vs outside of the center Drop Zone)

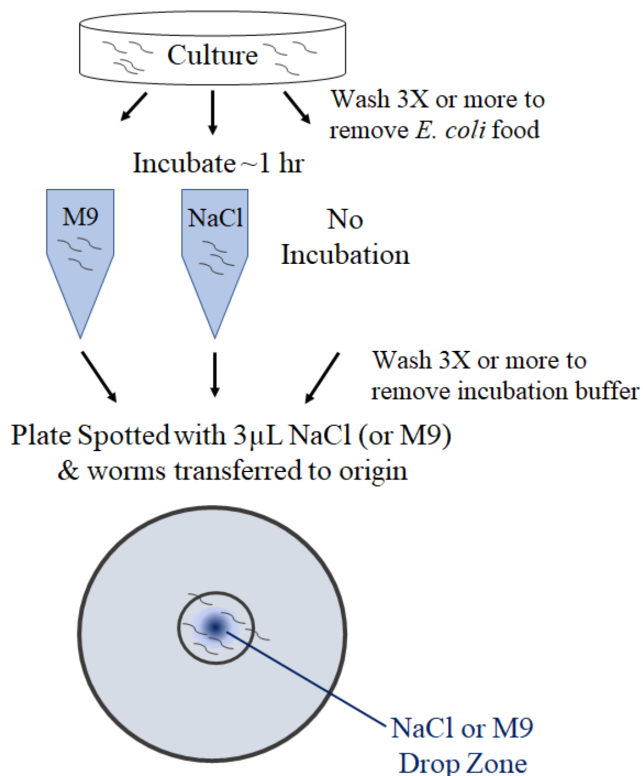

Figure 3. Associative Chemotaxis Protocol. Worm colonies washed from cultivation plates to ~1-hour incubation conditions, are then washed and collected to be placed in the test plate center origin where a 25 μL dry drop of 20 mM NaCl is located.

at ~2-minute intervals. In practice, pictures of associative chemotaxis test plates could be captured using a USB microscope (AM-313 Dino-Lite Plus Digital Microscope, Dino-Lite; USB2-micro-250X, Plugable) and counting worm placement with NemaCount (O'Halloran, 2016) making performing this particular assay possible without a dissecting microscope.

Individual Worm Mechanosensation Habituation Assay
Eyebrow Hair Pick: using wood glue a single eyebrow hair was attached to a toothpick. Hair picks were trimmed to increase strength (shorter is better for picking up and moving worms while longer hair picks are amenable for gentle head touch assays; Hart, 2006).

Procedure: Leica EZ4 dissecting microscopes were used to visualize individual worms and the shorter hair pick to move a single worm to a test plate (NGM plate with no food) giving 1 - 2 minutes for acclimatization. Using the longer hair pick, participants performed a prescribed series of head touch and tail touches to observe locomotor response changes. Participants then performed repeated head touch and repeated tail touch to demonstrate habituation.

Optogenetics
Microscope set-up: Orange transparent lighting filters (R23 Orange Cinegel Supergel, Roscolux) were added over all white-light sources on the dissecting microscopes to prevent additional blue wavelength light exposure to ChR2-expressing worms.

Procedure: Participants used short hair picks to move ChR2-expressing C. elegans to test plates. After 1 - 2 minutes of acclimatization to the test plate, high-intensity blue flashlights (1000 lumens CREE XM-L E17 Zoomable, CrazyFire) were employed to activate ChR2. One to two-second light pulses (durations sufficient to elicit a locomotor response) were to be delivered approximately every 30 seconds. Following repeated blue light exposure, participants were to deliver a head touch and observe the resultant locomotor response to determine if repeated activation of mechanosensory neurons had altered responsiveness to the head touch stimulus.

C. elegans Online Resources
Below is a list of publicly available online resources that provide additional details or information for developing experiments or performing described procedures:

WormBook – www.wormbook.org
and specifically, the Worm Methods Chapter - http://www.wormbook.org/toc_wormmethods.html
WormBase - https://wormbase.org/
WormAtlas - http://www.wormatlas.org/
SHIGEN National BioResource Project (NBRP)::*C.elegans* - http://shigen.nig.ac.jp/c.elegans/
Caenorhabditis Genetics Center - https://cgc.umn.edu/

METHOD HIGHLIGHTS AND RESULTS

Part I: Associative Chemoavoidance

C. elegans can associate a chemical attractant (e.g., NaCl) with aversive environmental conditions (i.e. starvation) and will show subsequent avoidance of that previously attractive substance (Saeki et al., 2001). To measure associative chemoavoidance, researchers typically condition worms by exposing to a high sodium chloride concentration (e.g., 20 - 100 mM) in the absence of their *E. coli* food source for approximately 1 to 4 hours (worms can be suspended in high NaCl-containing liquid buffer or transferred to high NaCl-containing agar petri plates, [Saeki et al., 2001; Tomioka et al., 2006]). During these incubation periods with no food, worms are thought to be forming an association between the NaCl and the absence of food, demonstrated during testing when a majority of worms avoid a high NaCl region on an agar test plate.

For the FUN workshop, a few time-savers were implemented to reduce variability. For instance, to perform an associative chemoavoidance task in the 2-hour time-period, worms were pre-incubated in the associative conditions (high NaCl and no food) prior to the workshop. With pre-incubated worm samples, participants did not have the experience of preparing worms for the associative incubation; however, preparing worms for incubation is almost identical to preparing worms for testing. Following the repeated washes, participants collected pelleted worms by capturing the bottom 10 - 15 μL and then deposited the worm pellet in the center of the test plate.

The chemoavoidance test condition was altered from the

typical assay described. For research, associative chemotaxis assay is most often performed by establishing a chemical gradient on agar-filled plates by placing a small agar plug containing high NaCl to one side of the plate; diffusion of the NaCl then occurs over hours (Hart, 2006). For a lab class where materials preparation may only happen for a few weeks in a semester/quarter, there seems a higher potential for variability and/or error in forming a consistent chemical gradient on agar plates. The following procedure was adopted whereby methods for associative chemotaxis were combined with the basic methodology of the dry drop test (Hart, 2006). This method relies on a relatively simple preparation and thus provides improved reliability with regards to testing associative chemoavoidance. Basically, instead of using an agar plate with a NaCl gradient, test plates are filled with agar absent of NaCl and then just prior to test a 20 µL drop of 20 mM NaCl is added to the center of the 9 cm test plate (the Drop Zone). To aid in visualizing exactly where NaCl was added, a drop of food coloring is added to the NaCl. Although NaCl and food coloring will diffuse at different rates, this provides an indicator of where the highest concentration of NaCl was initially. Once the NaCl drop is dry, testing is performed by simply adding the collected worm pellet from the incubation condition to the location indicated by the food coloring (i.e., the “Drop Zone”; see Figure 3). Because worms are added shortly after the drop of NaCl has dried, this produces a somewhat uniform chemical gradient as diffusion of NaCl should be minimal over the course of a few minutes.

For a typical associative chemotaxis assay, attraction is measured as a ratio of the number of worms that move towards the high NaCl area to the number of worms that move away from the high NaCl area on the NaCl-gradient test plate (Hart, 2006). There are a variety of ways to measure worm attraction/avoidance levels to NaCl with the Drop Zone procedure. For instance, one option is to record the average time it takes for a worm to “escape” the NaCl Drop Zone; Worms that do not associate NaCl with the absence of food will preferentially remain in the high NaCl Drop Zone. This data collection method requires continuous observation of the test plate which can be accomplished by video recording test trials. Alternatively, worms can also be counted inside versus outside the circle around the Drop Zone at specific time points following delivery to the test plate. For the FUN Workshop, participants ran two conditions (a control and an experimental group) and tallied the number of worms inside compared to outside of the Drop Zone circle at 2-minute intervals.

Using *C. elegans* for lab class experiments allows for manipulation of the well-understood neural circuits of the animal. Gustatory neurons are known to release serotonin (5-HT) in the presence of food (Sze et al., 2000). Serotonin also plays a role in learning a NaCl aversion (Hukema et al., 2008). An instructor can manipulate food-dependent signals pharmacologically by exposing worms to biogenic amines (e.g., 5-HT) or genetically by testing strains that carry a known mutation in a specific neurotransmitter pathway. For the FUN workshop, two extra experimental conditions were introduced. First, a third incubation condition was added where worms were exposed to both high NaCl and serotonin hydrochloride (1 mM; H5923, Sigma) in the absence of food. Second, a *ser-3* knockout strain was tested (codes for an octopamine receptor necessary for CREB-mediated response to starvation; Suo et al., 2006). In total there were 6 experimental conditions: adapted chemoavoidance assays were performed on WT or mutant worms (ser-3) that were incubated in M9 wash solution, NaCl or NaCl plus serotonin. Each FUN workshop participant ran one experimental condition and a WT control. It was intended that FUN participants would record the number of worms outside of the Drop Zone at different time points so a mean and error estimates for the different experimental groups could be calculated, but this was not possible due to workshop time constraints. However, anecdotally, participants noted differences in worm migration between the two conditions they tested as well as in comparison to other participants; the 5-HT exposed worms seemed to be the fastest to move away from the NaCl the *ser-3* mutants tended to stay in the center.

These manipulations that result in neurotransmitter-mediated alterations in behavior lend themselves to intriguing experimental questions that can easily be employed in a lab class. Figure 4 is an example of data collected by students in my lab class using this associative chemoavoidance assay. In this lab class, students performed RNAi knock down of *cam-1* gene expression (ROR receptor tyrosine kinase involved in Wnt signaling; Green et al., 2007) and used cost-effective USB microscopes (see Materials and Methods) to capture pictures and NemaCount (O’Halloran, 2016) for tracking worm locations and calculating avoidance indices. In the end, students calculated mean avoidance indices for each group (see Figure 4) and determined that both control and *cam-1* knockdown worms showed less attraction to NaCl following incubation with NaCl + no food. Although in this case the effects may seem subtle, the students benefited from performing a behavioral procedure that resulted in interpretable data and from which they could generate additional hypotheses for future research.

Part II: Touch Avoidance to Assess Individual Worm Behavior

In addition to group behavior assays like chemotaxis and thermotaxis, behaviors of individual worms are also well-documented. For instance, touching a worm's head or tail produces a relatively uniform locomotor avoidance response: worms will move away from the touch so head touch results in reversal responses while tail touch produces accelerated forward locomotion. Interestingly, there is plasticity within these reflexive responses as repeated head touches will produce a decrease in reversal probability and magnitude over time. This gradual decline in response due with repetition is not due to fatigue or sensory adaptation because a novel stimulus can typically return responsiveness suggesting that it was a plasticity process, known as habituation (Rankin et al., 1990). This form of learning is the simplest form of learning and all animals demonstrate the ability to habituate to redundant or repetitive unimportant stimuli in their environment. As habituation requires synaptic plasticity to occur, observing

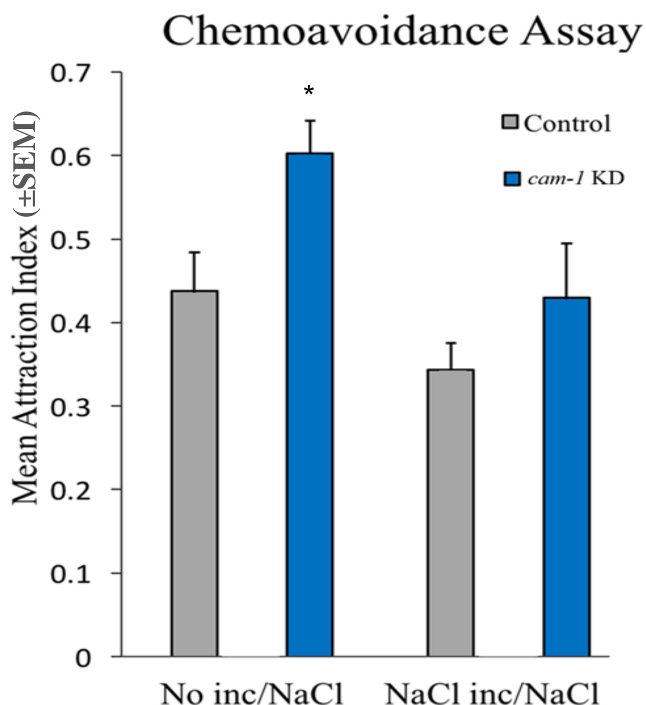

Figure 4. Sample data from an undergraduate lab class using the adapted chemoavoidance assay. Attraction index was calculated as: # of viable worms inside the Drop Zone/total # of worms for n = 8 test plates /condition. Data show a small decline in NaCl attraction for WT worms following NaCl incubation without food. Despite an overall greater NaCl attraction index, a similar decline in NaCl attraction is seen for *cam-1* knockdown worms. * = $p < 0.05$ determined by planned comparisons.

this gradual decline in behavior over time reveals something interesting about neuron signaling. Several reports have identified genes and signaling mechanisms important for this plasticity (Giles and Rankin, 2009). Thus, for a lab class it is an effective way to introduce synaptic signaling mechanisms and follow-up with students observing the behavioral outcome of changes in signaling.

The easiest way to introduce habituation to a touch stimulus in a lab class is to have students build their own "micro-contact devices" (i.e., eyebrow hair pick). This is a tried-and-true technique that has been used for several years in *C. elegans* research labs and requires only a toothpick some wood glue and an eyebrow hair (see Hart, 2006). Ideally participants build two; one where the eyebrow hair is left long (for gentle touch stimuli) and a second where the eyebrow hair is trimmed thus increasing the stability, this second, sturdier eyebrow hair pick can be used to literally pick-up individual worms or to deliver a "harsh" touch stimulus (Hart, 2006). Using the long eyebrow hair pick, head touch is performed by placing the eyebrow across the path of the forward moving worm or gently swiping the eyebrow hair across the head portion of the worm. Application of tail touch occurs in a similar manner.

A variety of methods can be employed to measure the worm's locomotor response to a touch stimulus. If using dissecting microscopes to observe behavior, the frequency with which a reversal response occurs following each touch can be noted, a simple Yes/No determination. As well, the approximate distance traveled can be estimated by eye (e.g., one wormlength, ½ wormlength, etc.). If using a USB microscope, behavioral observations can be recorded and analyzed later. The freely available ImageJ program offered by NIH (https://imagej.nih.gov/ij/) can also be used to analyze recorded behavior as specific macros can be imported for worm behavior analysis. A few downfalls of using an analysis program include: using analysis programs can be time-consuming, the recorded behavior needs to be captured at a higher resolution and use of USB microscopes makes it difficult to accurately deliver head/tail touch. Measuring forward or backward movement in *C. elegans* can be as simple or as complex as process of a process as needed depending on the learning goals of the lab class.

Reviewing the neural circuitry and signaling involved in *C. elegans* locomotion and then watching worms respond to simple touch stimuli is intriguing; however, it is also of interest to monitor how the behavioral response decreases with only a few repetitions of the touch stimulus (habituation). To apply a touch stimulus in a somewhat uniform manner, students should time the intervals between touch stimuli. If students wait a short duration between touches, for example 10 seconds between each touch stimulus, a dramatic and rapid decrease in responding should occur within 3 - 5 stimuli. This can be compared to a longer interstimulus interval (e.g., 60 seconds) between each touch stimulus in which case the decline in responding will be less visibly obvious compared to the shorter interstimulus interval. In addition to habituation, other elements of this plasticity can also be tested easily in a laboratory class. For instance, after habituating an animal to a head touch stimulus one could have participants gently blow on the agar plates containing their worms and then re-examine the responsiveness of the worms again to head touch. The breeze generated by lightly blowing on the plate likely causes worms to dishabituate, meaning their response to head touch would return to initial response levels.

The neural mechanisms for habituation can be discussed in the context of the behavioral results. Again, mutant strains, RNAi knockdown of identified genes or exposure to chemicals or drugs can be employed to further investigation neural mechanisms for habituation in a lab class. For instance, the *eat-4* glutamate mutant (carrying a mutation for the glutamate transporter thus affecting the availability of the neurotransmitter glutamate; Lee et al., 1999) shows rapid habituation compared to wild-type (Rankin and Wicks, 2000; Rose et al., 2001). Further, worms tested on food show a different rate of habituation than worms tested off food (Kindt et al., 2007). This seems to be due to release of dopamine as a result of the food exposure and brings in an additional element with regards to polysynaptic signaling. Finally, a number of pharmacological agents can also be employed including psychoactive drugs, toxins, etc., provided exposure to the agent does not interfere with locomotion. Thus, measuring behaviors to examine habituation in a laboratory class provides many avenues of investigation to demonstrate the connection between synaptic signaling and behavior.

Part III: Inducing Neuron Activity with Optogenetics and Observing Behavior

In my lab classes, students typically view the use of optogenetics as exciting or "cutting edge" and are fascinated by the processes by which channelrhodopsin (and other opsins) function. Capitalizing on student interest and enthusiasm for this topic by introducing optogenetics in a lab class further supports their understanding of neuron depolarization and hyperpolarization in a sophisticated way. By employing transgenic *C. elegans* strains that express a channelrhodopsin gene (e.g., ChR2) within an identified neural circuit, students can activate directly synaptic signaling and observe the behavioral outcome.

Activation of channelrhodopsin can be achieved by exposing worms to blue light of sufficient intensity (~1mW/mm2; Nagel et al., 2005) and as *C. elegans* are transparent, no sample preparation is required. Today, researchers have used LEDs to activate either channelrhodopsin or halorhodopsin (Grossman et al., 2010; Singaram et al., 2011; Ardiel et al., 2017). For lab classes and the FUN workshop, high-intensity blue light flashlights (see Materials and Methods) were used as they are cost-effective, durable and sufficiently bright to activate channelrhodospin (a blue light flashlight source measured at a 26cm distance can emit ~2mW/mm2 and sufficiently illuminates the surface of a 60mm agar-filled petri dish; see Figure 5). To help eliminate extraneous blue light, white lights used for viewing under a microscope are covered with an orange colored transparent filter (see Materials and Methods) allowing students to restrict ChR2 activation to periods of observation. Similar to *Drosophila*, *C. elegans* require ATR to form functional channelrhodopsin channels; thus, channelrhodopsin strains must be pre-exposed to ATR by adding ATR to their bacterial food source (Nagel et al., 2005). Channelrhodopsin worm stocks should also be protected from light (plates containing ChR2 worms are wrapped in aluminum foil).

When considering *C. elegans* ChR2 for a lab class, there is one important caveat to note: the worm has an endogenous photoreceptor that mediates an avoidance response to blue light (Edwards et al., 2008; Ward et al., 2008; Gong et al., 2016). The avoidance response is described as a protective measure to prevent DNA damage should the worm be exposed to long wavelength light (i.e., UV, Blue). Because of this, many ChR2-containing *C. elegans* strains are expressed in a *lite-1* mutant background to limit the endogenous response to blue light. If using a ChR2 strain not expressed in a *lite-1* mutant background, one possible way to account for the contribution of the endogenous light response would be to compare responses of ChR2 worms cultivated either on or off ATR (ATR+ vs ATR-). Any light-elicited responses in the ATR- group would presumably be due to the endogenous response.

For the FUN workshop, a transgenic strain that expressed ChR2 in the mechanosensory neurons (pmec-4::ChR2; see figure 2) in a *lite-1* mutant background was employed. When all of the mechanosensory neurons are activated together by a non-localized mechanosensory stimuli, this generates a reversal response (Chalfie et al., 1985; Wicks and Rankin, 1995). Thus, it would be expected that when the mechanosensory neurons become depolarized through the activation of channelrhodopsin expressed in these neurons, this would also result in a similar reversal response. In the lab class, participants noted worms were typically reversing when exposed to blue light.

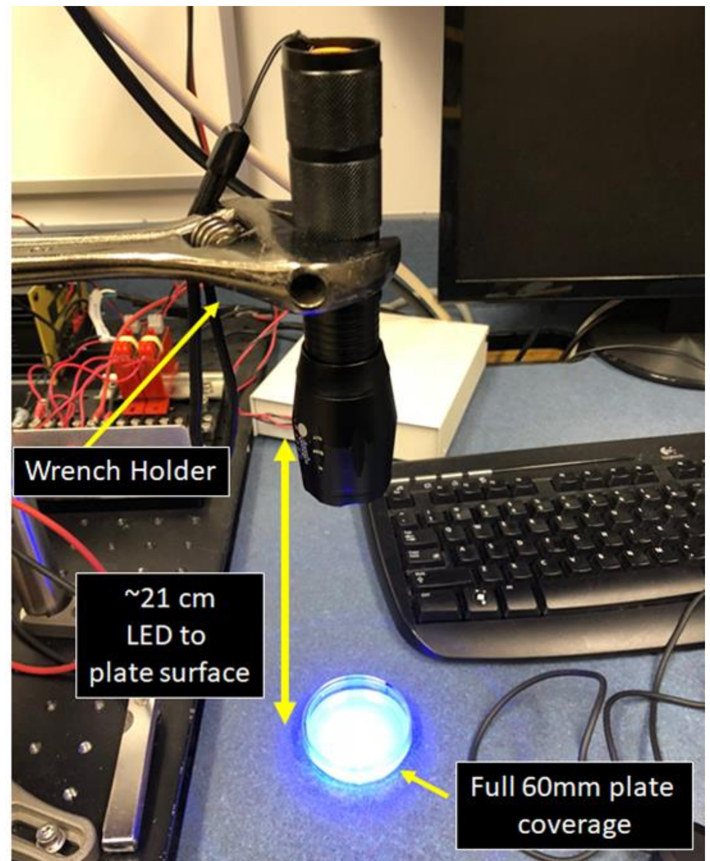

Figure 5. Blue Flashlight setup. Sufficient light intensity to activate ChR2 (~2mW/mm2) was measured from these devices at ~21 cm from the plate surface, a distance that covered the plate area.

Employing optogenetics in a lab class, allows students to test unique experimental questions. For instance, it has been reported that habituation results from repeated ChR2 activation of a nociceptive neuron (ASH) and that this is modulated by the dopamine receptor, DOP-4 (Ardiel et al., 2016). Expanding on this, in one of my lab sections students examined the behavioral effects of repeated ChR2 activation of inhibitory GABAergic motor neurons using an $_p$*unc-47*::ChR2 strain (see Figure 2; although not noted, *unc-47* is also expressed in chemosensory and thermosensory circuits). Lasting behavioral effects of repeated blue light exposure were examined by measuring reversal speed following head touch (Figure 6). Further, students in this class investigated if CAM-1 mediates any possible behavioral effects of repeated *unc-47*::ChR2 activation (in adult worms Wnt signaling occurs at the neuromuscular junction; see Jensen et al., 2012). From the results, students interpreted that repeated light-induced activation of ChR2 in GABAergic motor neurons produced a small decrease in reversal speed to head touch while *cam-1* knockdown had seemingly the opposite effect (Figure 6). In this example, students performed a genuine experiment trial

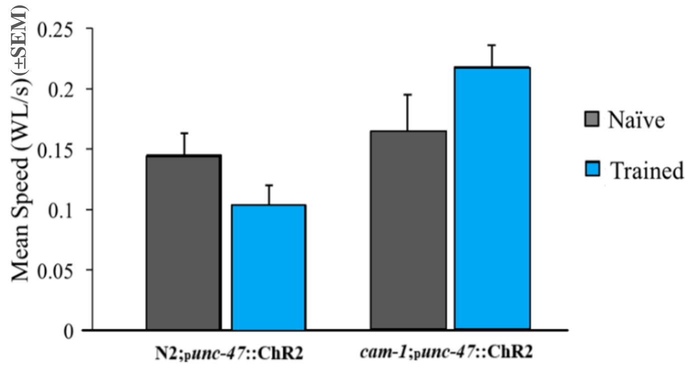

Figure 6. Sample data from an undergraduate lab class. Behavioral effects of repeated ChR2 activation of GABAergic motor neurons (p*unc-47*::ChR2). Behavior was measured as approximate speed of reversal responses resulting from head touch (n = 8/condition). Worms that underwent *cam-1* RNAi knockdown show increased responsiveness to head touch after repeated p*unc-47*::ChR2 activation (5 s blue light exposure every 60 s for 5 min).

This workshop met my expectations

Response Number; 0, 5, 10, 15, 20, 25

Strongly Disagree, Disagree, Neutral/Unsure, Agree, Strongly Agree

Response Options

This workshop increased my understanding of the topic material

Response Number; 0, 5, 10, 15, 20, 25

Strongly Disagree, Disagree, Neutral/Unsure, Agree, Strongly Agree

Response Options

The exercise presented at this workshop is a good vehicle for teaching undergraduates prinicples of neuroscience

Response Number; 0, 5, 10, 15, 20, 25

Strongly Disagree, Disagree, Neutral/Unsure, Agree, Strongly Agree

Response Options

This workshop was well-organized, with clear objectives

Response Number; 0, 5, 10, 15, 20, 25

Strongly Disagree, Disagree, Neutral/Unsure, Agree, Strongly Agree

Response Options

I am likely to incorporate the material in this workshop as a classroom activity

Response Number; 0, 5, 10, 15, 20, 25

Strongly Disagree, Disagree, Neutral/Unsure, Agree, Strongly Agree

Response Options

The exercise presented at this workshop is a good vehicle for teaching undergraduates prinicples of neuroscience

Response Number; 0, 5, 10, 15, 20, 25

Strongly Disagree, Disagree, Neutral/Unsure, Agree, Strongly Agree

Response Options

Figure 7. Histogram data of faculty assessment of two-hour FUN workshop. One assessment was omitted as individual rated 'strongly disagree' on all six items along with the following comment: Awesome! Can't wait to work with worms. Total number of participants across four workshop presentations = 29.

that resulted in original data from which they could generate additional hypothesis to base future experiments. Students presented the results of their lab class research at both Department- and University-wide research conferences.

Part IV: Workshop Ratings

From the FUN workshop, faculty participants rated the overall workshop highly with regards to clarity, organization and meeting expectations, and recognized that these adapted behavioral assays could provide a good foundation to teach general principles of Neuroscience (see Figure 7). There was more variability in the number of faculty participants who would be likely to incorporate this material in their own classes or use the material presented as a resource for class lectures or their own Neuroscience background. Many faculty noted that this was their first experience with *C. elegans.* Respondents who rated 'disagree' for incorporating the workshop material in their teaching commented that 'their courses focus on human behavior' and 'inappropriate for my course but will recommend to a colleague for their lab class.''

CONCLUSIONS

The *C. elegans* model system offers several avenues to provide an authentic research experience within the time and resource constraints of a lab class. The assays outlined above demonstrate that with a few procedural modifications, popular learning assays can be employed in a lab class to demonstrate the connection between neuron function and behavioral outcome.

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